CHAPTER ELEVEN

Bacterial Transformation

> It may very properly be asked whether the attempt to define distinct species, of a more or less permanent nature, such as we are accustomed to deal with amongst the higher plants and animals, is not altogether illusory amongst such lowly organized forms of life as the bacteria. No biologist now believes in the absolute fixity of species. . . . But there are two circumstances which here render the problem of specificity even more difficult of solution. The bacteriologist is deprived of the test of mutual fertility or sterility, so valuable in determining specific limits amongst organisms in which sexual reproduction prevails. Further, the extreme rapidity with which generation succeeds generation amongst bacteria offers to the forces of variation and natural selection a field for their operation wholly unparalleled amongst higher forms of life.
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> (Andrewes, 1906, 14)

> When confronted on every hand with such pictures of bacterial instability . . . it is logical, first to inquire whether the confusion we observe is pure chaos, or whether there exists any trace of orderliness amidst the general disorder. . . . Cocci become rods and rods cocci or spirals; forms of growth change overnight; motility is lost or regained; fermentation reactions are modified by time and opportunity; spore formers become sporeless; haemolytic activity comes and goes; capsulated bacteria lose their capsules, and capsules are gained by noncapsulated forms; antigenic power vanishes and reappears; cultures become spontaneously agglutinative or fail of agglutination; virulent cultures become harmless and harmless cultures virulent.
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> (Hadley, 1927, 5)

In the 1870s there was considerable scepticism on the subject of bacterial species. The botanists, Carl Nägeli and Ferdinand Cohn denied their existence (Cohn, 1875; Nägeli, 1877) but Robert Koch affirmed it and he won the day. His doctrine of constant bacterial species was first undermined by studies of a variable strain of the colon bacillus known as *mutabile* (Neisser, 1906; Massini, 1907), and their studies were soon supported on the basis of the single-cell culture technique. This work was rapidly followed up by other workers. "The result" wrote Hadley, "was to bring into the field of study of bacterial variation the de Vriesian term 'mutation', imported from the botanical literature" (Hadley, 1927, 10). Inevitably, the whole question of bacterial variability was open once more, and evidence was not lacking. Thus, changes in the ability to ferment specific media and attenuation of virulence were observed at the end of the nineteenth century. Roger, at the Pasteur Institute, observed what was probably a spurious case of attenuation in pneumococcal cultures in 1891, later, more definite evidence was obtained by Neufeld at the Robert Koch Institut, Berlin, in 1902. But what was

the significance of these changes? Were they lasting changes like de Vries' mutations, or were they equivalent to his "fluctuating" variations? An understanding of such changes, it was hoped, would help to explain the fluctuations in the severity and incidence of diseases. Such was the background to Griffith's discovery of bacterial transformation in pneumococcus.

The Discovery of Rough and Smooth Forms

The British bacteriologist, J. A. Arkwright, at the Lister Institute, London, studied the characteristics of the virulent and attenuated strains of several bacteria, chiefly Shiga's bacillus. In 1921 he gave a clear description of their colonies, the virulent ones being smooth, dome-shaped, and regular; the attenuated ones being granular, flat and irregular. He introduced the terms rough R and smooth S, and described them as persistent variations or "mutants" (Arkwright, 1921, 55). Since the R forms were only observed under artificial conditions, Arkwright saw that the observed uniform character of bacteria like *B. typhosus* and *B. dysenteriae in vivo* was due to selection. "The human body infected with dysentery may be considered a selective environment which keeps such pathogenic bacteria to the forms in which they are usually encountered" (*Ibid.*).

Arkwright had picked out an important example of microbial variation. He had shown that, once produced, the R-form was reproduced faithfully in the subsequent colonies subcultured weekly, but above all he had given very clear and simple criteria for distinguishing the two forms visually. Small wonder then that his work was "seized upon at once, first and foremost by the English school" (Hadley, 1927, 12). The R and S forms were described in streptococci in 1922, pneumococci in 1923, *B. enteritidis* in 1924 and Salmonella in 1925. At the Rockefeller, Paul de Kruif studied the S and R forms in the bacillus of rat septicaemia. In London, Frederick Griffith demonstrated reversion of R to S in pneumococcus, by animal passage and in plate culture (1923), and this was confirmed by Levinthal working in Berlin under Neufeld (1926).

The R and S Forms of Pneumococcae

The extension of Arkwright's R and S forms to pneumococcus was achieved by Griffith, a quiet and retiring medical officer of health at the Ministry's pathology laboratory in Endell Street, London. He was the most English of Englishmen. "He was a civil servant and proud of it. He had that kind of a mind and the integrity that often goes with it. He did not allow his fancy to roam . . . and being employed by the Ministry of Health to do a specific job, he believed in fulfilling his contract however frustrating that might be" (Elliot, 1970). Allison has described just how retiring Griffith was: vain were all attempts to persuade him to attend the meetings of the Pathology Society

and the Royal Medical Society, or to read a paper to the Medical Research Club. To get him to the International Congress for Microbiology to hear Rebecca Lancefield talk on streptococcal types Allison and Scott had to order a taxi and bundle Griffith into it (Allison, 1969; Pollock, 1970, 7). No wonder that the great immunochemist, Avery, never came to know Griffith personally and never corresponded with him (Pollock, 1970, 10).

The problem that the Rockefeller Hospital scientists were trying to solve was the production of an effective immune serum for treatment of patients suffering from acute lobar pneumonia. Long ago Neufeld, at the Robert Koch Institute, had classified pneumococci serologically into three types, and had thus laid the basis for the recognition of Types I, II and III. The Rockefeller groups added a fourth Type which, because of its heterogeneity, became known as the "American Scrap Heap". Griffith renamed this collection "Group IV".

Neufeld observed that Type I pneumococcae were the commonest to be found in cases of pneumonia brought to his attention in Berlin. Therefore he called this type "typical" in contrast to the other two types which he called "atypical". He suggested that in order to produce a successful immune serum an attempt should first be made to prepare one against Type I pneumococcae, rather than against the "atypical" type. The Rockefeller group and Griffith also found Type I most common. They set to work and prepared an immune serum, but as Griffith's colleague, Dr Arthur Eastwood wrote, it was far from an unqualified success.

> Whilst appreciating the value of the progress which has been made, it must be admitted that the present position is unsatisfactory. The Rockefeller investigators can only offer serum therapy if the case is found to be due to Type I; in that event, their experience is that large and repeated intravenous injections of specific serum will bring down the mortality due to this type from about 25 to about 10 per cent. Diagnosis of the type should be made at an early stage of the disease and treatment should follow immediately. But, in hospitals, patients are often in an advanced state on arrival; and, owing to the special skill and care which are required and to the very large quantities of serum needed, this treatment is not likely to be readily adopted by the general practitioner.
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> If the precise antigenic characters of the infecting strain of pneumococci are all important, one can understand that it would be difficult, if not impossible, to provide therapeutic sera which would be useful for infections with "atypical" strains; but, even on this assumption, there is no generally accepted explanation why immunization of horses with the second and third of the "fixed" types has failed to produce a good therapeutic serum, when immunization with the first has succeeded.
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> (Eastwood, 1922, 18–19)

What a contrast was pneumococcus with the straightforward behaviour of diphtheria and smallpox! It threw up problems which called for a very thorough knowledge of its antigenic properties. Had it not proved so difficult the science of immunochemistry would surely not have been so strongly promoted, and the transformation of pneumococcal types might never have come to occupy so important a role in the identification of the hereditary

material! One thing the experiences at the Rockefeller Hospital taught Avery and Dochez was just how distinct were the various types of pneumococcus. Their study of the immunity reaction, on the other hand, showed them that, like diphtheria, live pneumococci do secrete soluble substances into the host organism, and that the quantity liberated seemed to be related to the amount of capsular material around the pneumococcus—much in Type III and least in Type I. Although the evidence pointed in another direction they considered the soluble substance to be "of protein nature or to be associated with protein" (Dochez and Avery, 1917, 493).

Five years later Avery was given something of a jolt when an extract like his specific soluble substance was obtained from a number of bacterial species, including pneumococcus, and shown to be protein-free (Zinsser and Parker, 1925). This work had been carried out at a rival institute—the College of Physicians and Surgeons in New York—and its authors suggested that they were dealing with an antigen composed of two parts, the one a nucleoprotein which stimulated antibody production, the other a non-protein "residual antigen" which reacted with the antibody. The latter they speculated might be an example of the hypothetical hapten molecules of which Landsteiner had written (Landsteiner, 1919).

Then it was Avery's turn to show that the specific soluble substances in pneumococcus were unusual polysaccharides and that the capsular carbohydrate, long known, was in fact involved in the determination of serological activity (many papers starting with Heidelberger and Avery, 1923; Avery and Heidelberger, 1923).

What had been a series of serological types now became in addition a set of chemically distinct types.

Change of Pneumococcal Types and the Onset of Acute Pneumonia

When in 1923 Griffith discovered the S and R forms in pneumococcus and their interconversion *in vivo* and *in vitro*, the Rockefeller concept of type specificity was not *directly* challenged, for reversion of R to S forms always led to the production of a specific soluble substance identical with the S type from which the R form had originated. But Griffith was exploring a more daring suggestion. He knew that associated with the R and S forms was the property of non virulence and virulence respectively. He knew furthermore that the Rockefeller scientists had shown Types I and III to be associated with acute lobar pneumonia whereas members of their "Scrap Heap"—Group IV—were found in the sputum from healthy individuals and in patients recovering from acute lobar pneumonia. Whereas the Americans simply concluded that Types I and II died out during convalescence and were replaced by Group IV, which was not invasive and therefore remained in the mouth, Griffith as early as 1922 wrote:

> An alternative theory is that the virulence of Types I and II becomes attenuated during convalescence, and this change is accompanied by mutation of type characters, which now become degraded into those of the heterogeneous and less virulent group termed IV. There are two experimental difficulties about this view. . . . Still it is theoretically possible that mutation may occur in nature, though it cannot be reproduced *in vitro*.
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> (Griffith, 1922, 35–36)

To test for such an event he sought for different serological types from one and the same patient during the course of the disease and he found them.

> If mutation occurred, one might expect some regularity in the serological characters of the strain which replaced the Types I and II. . . . Although many of the infections have been apparently pure, it is very striking how often a typical bile soluble diplococci can be found in sputum, even during the acute stage, together with the Types I and II. . . .
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> (Griffith, 1922, 36)

From the beginning, it was Griffith's desire to show affinities rather than differences between the pneumococcal types.

> The various races of pneumococci resemble each other so closely in appearance of colonies and in the characteristic of bile solubility that there can be no doubt that they belong to one species.
>
> (*Ibid.*)

When he introduced his discovery of S and R forms in pneumococcus he wrote: "The conception of a 'pure culture' of a bacterium as a number of absolutely identical individuals is no longer tenable" (1923, 1). Griffith seems to have felt that mutation *within the limits of the species* was acceptable since it was a device by which the species adjusted itself to changes in the environment. It was a "natural tendency with many species of bacteria" (Griffith, 1923, 11). The S to R change was one example; it was "attributed to degenerative changes" and was "associated with the loss of certain antigenic qualities" (*Ibid.*). Because it was achieved regularly when immune serum had been added to the medium Griffith suggested that the serum not only sensitized the bacteria in preparation for phagocytosis (Neufeld's bacteriotropic theory) but it caused those which escaped this fate to become rough and therefore non-virulent (Griffith, 1923, 12–13).

We can, I believe, conclude that Griffith would have denied the transformation of one bacterial species into another, but for him the pneumococcal types and R and S forms were mutable characteristics *within* the species. Such "mutations" were distinct from the important mutations discovered by de Vries. In this Griffith sided with P. Hadley who felt it was not in accord with biological principles

> that variations of hereditary significance (that is, true mutations) would be formed as easily or as commonly as we observe to be the case . . . or that micro-organisms in general are addicted to discarding permanently their ancient hereditary characters with such apparent nonchalence . . .
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> (Hadley, 1927, 224)

Griffith's Discovery of Transformation of Types

From Avery's point of view the story took an unexpected and regrettable turn when in 1928 Griffith reported the transformation of the pneumococcal types which Avery and Heidelberger had so firmly established as invariable. The story seemed improbable therefore from the beginning, but even more so when the method by which Griffith had achieved this transformation was considered. He had injected the living cells of the R form of Type I pneumococcus into a mouse, together with heat-killed cells of the S form of Type II. The mice succumbed to the infection and died. From their blood Griffith isolated colonies of the S form of Type II! Not only had there been a reversion from R to S but a *change of type*. The capsular substance of the colonies isolated was identical with that of the dead Type II cells, and not of the capsular type from which the living R cells had been obtained by serial sub-culture.

If this phenomenon could have been simply a question of the dead polysaccharide coats being used by the living cells Griffith's result would have been considered more plausible, but not only did the polysaccharide belong to a different pneumococcal type from that of the living R cells but it was resistant to steaming, yet the dead S cells lost the power to transform the living R cells if they were heated above 80°C. For Griffith then, the transforming substance had to be thermolabile; the polysaccharide was thermostable. He called it S substance and wrote: "By S substance I mean that specific protein structure of the virulent pneumococcus which enables it to manufacture a specific soluble carbohydrate" (1928, 151).

Now, he argued, how could the Type I cell utilize the S substance of a Type II cell unless it already contained some of the S antigen of Type II. Here he seemed to slip from S substance to S antigen and thus made his meaning ambiguous. But for Griffith, as for Avery, the S antigen was a compound structure *not* equivalent to the soluble specific substance. Otherwise the latter would be found capable of stimulating antibody production, but it was not (Zinsser and Parker, 1923; Avery and Heidelberger, 1923). It was therefore quite consistent for Griffith to speak of utilizing the S antigen from dead cells. He wrote:

> Since virulence and the capacity to form soluble substances are attributes of the S strain, their possession may for convenience be ascribed to a special antigen which may be termed the S antigen.
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> (Griffith, 1928, 149)

His argument was, then, that an R form of Type I may possess a rudiment of the antigenic protein required to make the specific soluble substance of a Type II S form. All it required was more of this protein in order to function effectively. Now the transformation of RI to SII became in Griffith's eyes not so very different from ordinary reversion of RI to SI. In the latter pro-

cess the S antigen "remaining in an R strain may be regenerated and reach its original abundance under suitable conditions" (*Ibid.*, 152), as when he innoculated the R form with heat killed dead cells of the S form of the *same* type, or when the R form multiplied in a protective nidus under the skin and the cells which survived the host's reaction utilized the remnant of the S antigenic protein from those pneumococci which had not survived attack. Now suppose such an R cell "may contain in addition to its major antigen a remnant of the other type antigen" (*Ibid.*). What then was the difference between the R form of Types I, II and III and Group IV? For Griffith there was little if any difference.

> When pneumococci of Types I and II are reduced to their respective R forms by growth in homologous immune sera, they lose nearly all their major S antigen though they may retain their minor S antigens which are presumably not affected by the heterologous immune substance. But the major S antigen apparently still preponderates, since an R strain on reversion to the S form regains its original type characters.
>
> (*Ibid.*)

Griffith went on to point out the fact that in those R strains of Type I which did not revert spontaneously to their S forms there was no such preponderance of the residual Type I S antigen and hence there was no difference between the R form of these strains and that of a Type II pneumococcus! Then he waxed bolder and suggested that between Types I and II "there is no essential distinction".

> In fact, there are certain indications that the R pneumococcus in its ultimate form is the same, no matter from what type it is derived; it possesses both Type I and Type II antigens in a rudimentary form or, as it may be differently expressed, it is able to develop either S form according to the material available.
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> (Griffith, 1922, 153)

If by "is able to develop either S form . . ." Griffith meant that the R form had the protein required to synthesize either type, then the following oft-quoted passage makes more sense:

> When the R form of either type is furnished under suitable experimental conditions with a mass of the S form of the other type, it appears to utilize that antigen as a pabulum from which to build up a similar antigen and thus to develop into an S strain of that type.
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> (*Ibid.*)

This idea of the pabulum jars on our ears, accustomed as we are to far more explicit statements of the relation between genetic constitution and its expression in the visible characteristics of the organism. It may help, therefore, to understand Griffith's thinking if we turn to the report which his much more speculative colleague, A. Eastwood, wrote for the Ministry of Health in 1923. At that time transformation of pneumococcal types had not yet been discovered, but bacterial variation was well known, especially Arkwright's S to R change, and Griffiths' observation of it in pneumococcus, together

with reversion of R to S. Eastwood belonged to the orthodox school who, as we saw in the last chapter, adopted the enzyme theory of life. For him there were two phases in the life cycle of a bacterium: (1) Catalytic, or as we would say catabolic; (2) Synthetic, that is, the building up of new protoplasm. In the former the union of enzyme with substrate was transient, after which the digested substrate was liberated, in the latter the union of enzyme with substrate was stabilized and the resulting complex yielded protoplasm with all its properties, such as ability to manufacture the specific soluble substance. ". . . bacterial protoplasm may be regarded as a complex of enzymes and the products of enzyme action, a complex which involves the synthesis of these products (e.g., amino acids) into proteins" (Eastwood, 1923, 19). He argued further that there existed a "critical phase" in the life of the growing bacterial cell when a transition from catalysis to synthesis occurred. This was a delicate time when external influences could easily upset the balance and initiate fresh formation of protoplasm before all the ingredients from the environment were available. Defective protein would result. Or the catalytic stage might be prolonged and bacterial lysis would result. (Eastwood belonged to the Bordet/Gratia School and denied the existence of d'Hérelle's bacteriophages.) And what happened when the S form of pneumococci grew in a culture containing immune serum?

> In this case it is reasonable to postulate that, when digested food particles are being synthesized into protoplasm, the antibodies find in some of those particles their appropriate antigen and "pick them off"; the new bacterium is synthesized, but it is an impoverished bacterium (a variant), because it has been robbed of some of its antigenic components.
>
> (Eastwood, 1923, 21)

Eastwood's conception of metabolic activities was clearly cast in the mold of nineteenth century chemical vitalism. We find references to the "side chains" and "active groups" of protoplasm which remind us of Ehrlich and of the complex protoplasmic molecule. Finally, in the foreword to Griffith's and Eastwood's reports we find George Newman using the word "pabulum" (Newman, 1923, iv). This takes us back to the days when enzymes were chiefly known for their catabolic roles and when the distinction between growth and replication was not recognized. It shows us too, how long-lasting in some quarters was the vitalistic conception of protoplasmic synthesis as originally detailed by Pflüger in 1875.

Griffith's Interpretation of his Results

It has been stated recently that Griffith's demonstration of type transformation "must surely have been made almost *despite* his own emotional inclinations, rather than, as is so often the case, because of them" (Pollock, 1970, 7). We have seen that there are good reasons for believing that almost the converse of this was true, and that in 1922 and 1923 Griffith was toying with the

idea that within the "species" there are characteristics like the type of polysaccharide capsule *subject to mutation in response to environmental conditions.* Admittedly, he had no evidence of type mutability from plate cultures, but he did have the observation of more than one type in the same patient. Was this due to multiple infection or to mutation? The work described in his 1928 paper was aimed at deciding between these two explanations. Having shown that mutation can be produced experimentally Griffith opted for this alternative, and explained it in nineteenth century Darwinian terms.

Like Darwin and the nineteenth century animal breeders, Griffith pictured the progressive "fixing" of characteristics and he asked whether, if transformation of type "is a question of altered environment, are the influences which initiated the divergence of type still at work, i.e., are the type characters still in a state of flux, or have the different varieties become stabilized?" (Griffith, 1928, 148). Well, he showed that they were indeed still in a state of flux. The S form of Type II stimulated the production of a "specific immune substance" by the host which caused clumping together of the pneumococci when they became susceptible to phagocytosis.

> By assuming the R form the pneumococcus has admitted defeat, but has made such efforts as are possible to retain the potentiality to develop afresh into a virulent organism. The immune substances do not apparently continue to act on the pneumococcus after it has reached the R stage, and it is thus able to preserve remnants of its important S antigens and with them the capacity to revert to the virulent form.
>
> (1928, 156)

The bacterium did not necessarily play a purely passive part in the host–pathogen relationship, "the various forms and types may be assumed by it to meet alterations in its environment" (*Ibid.*).

In contrast to Griffith, Arkwright had presented a view of bacterial mutation which was modelled on de Vries' mutation theory, but even Arkwright was worried by the widespread occurrence of the same mutation S → R. And after Griffith had introduced the idea of major and minor antigens, Neufeld and Levinthal happily repeated it, and Dawson and Alloway explicitely supported it.

The Significance of Transformation for Epidemiology

We have already seen that the Rockefeller Hospital staff found Group IV pneumococci rarely in association with acute lobar pneumonia in contrast to Types I, II and III. Griffith considered it likely that Group IV represented the non-invasive type which could survive in the upper respiratory tracts without causing the onset of disease. From this situation it could spread by aerial infection to other hosts. To become invasive it "evolved" into Type I, II or III, reached the lower tracts of the lungs and there brought about acute lobar pneumonia. Should the host recover, the pathogen simply changed

back into Group IV and survived in the upper respiratory tracts of the convalescing patient.

But what of epidemics of pneumonia? Could these be accounted for in terms of transformation of type? It is clear that Griffith had hoped from the outset that some such mutation did lie behind the spread of pneumonia through a population. He had been impressed by the fall in the frequency in Smethwick of Type II from 32.6 per cent of the cases in 1920 to 7.4 per cent in 1927. This was paralleled by a rise from 30 per cent to 53 per cent in the frequency of Group IV. Surely this change had resulted from transformation of type? We can now see Griffith's work in its context. To learn how to control the incidence and spread of chronic lobar pneumonia called for a detailed knowledge of the host–bacterium relationship. Griffith believed he had shown there was more to this relationship than was generally believed. When bacteria were killed by the host their presence along with living bacteria did not merely negate the action of leucocytes by the "aggressin" popularly held to be liberated from them. The living R cells "actually make use of the products of the dead culture for the synthesis of their antigen" (Griffith, 1928, 150). And as his experiments showed, this could lead either to reversion of R to S of the same type or to that of another, since all pneumococcal types retained a rudiment of the protein structure necessary for making *any* of the various polysaccharide coats.

What an unfortunate turn events were taking in the world of microbiology! The extreme position of Robert Koch and F. Cohn, who believed in the fixity of bacterial types, had already been undermined. Now Avery's and his colleagues' demonstration of the constancy of pneumococcal types appeared to be going too. The opposite position from Koch and Cohn, represented by Carl Nägeli, who asserted the interconvertibility of bacterial species (1877), seemed in danger of coming back into fashion. Griffith's work was not just an oddity that could be shrugged off; it was a bombshell which fell into a fused situation, and Avery had every reason for *not* accepting it. Griffith's reputation was high, but his 'Lamarckian' ideas and his vague talk of a "pabulum" could hardly have appealed to Avery, who, though cautious to the point of conservatism, was at least committed to strictly chemical explanations. Small wonder, then, that he was not the prime mover behind the repetition of Griffith's experiments at the Rockefeller.

The Confirmation of Griffith's Results

At the Robert Koch Institute in Berlin, Neufeld and his assistant Levinthal were so quick to repeat Griffith's work that their confirmation of his results appeared in the same year, 1928, as Griffith's own paper. This had been possible because Levinthal had already been working on bacterial variation, having achieved reversion of R to S pneumococci without change of type in

1926, and also because Neufeld had visited Griffith's laboratory while the transformation studies were in progress and therefore knew the details (McCarty, 1968; Neufeld and Levinthal, 1928, 324). In 1929, in far-off Peking, H. A. Reimann also confirmed Griffith's work. But it was the fact that so reliable a bacteriologist as Neufeld has been able to reproduce bacterial transformation that made urgent the repetition of Griffith's work in the Rockefeller itself. This was done not by Avery but by the strongly pro-British Canadian, Henry Dawson, who "took advantage of the fact Avery had to be away for more than six months (because of hyperthyroidism) to repeat and confirm Griffith's experiments" (Dubos, 1972; Dawson, 1929). In his biographical memoirs of Avery, Dubos wrote: "For many months, Avery refused to accept the validity of this claim and was inclined to regard the finding as due to inadequate experimental controls. This scepticism was understandable in one who had devoted so much effort and skill to the doctrine of immunological specificity" (Dubos, 1956, 41). It was as if history was repeating itself, just as the firm ground won by Robert Koch was undermined, so was that of Avery!

According to George Corner, Avery had "asked Dawson to look into Griffith's transformation" (Corner, 1964, 461). This seems unlikely since it was never Avery's custom to ask anyone to undertake a specific piece of research. Had Avery played any part in the confirmatory work one would expect there to be some reference to him in Dawson's two papers of 1929, but there is none. Avery's name is on neither paper, yet when Dubos isolated a substance capable of digesting the capsular polysaccharide of pneumococcus while Avery was on holiday in the summer of 1930, Avery's name went first on the paper published by *Science* in August. Corner gives no source for his information. Dubos was there at the time. Dawson's confirmation of transformation by subcutaneous injection was reported in his second paper, received by the *Journal of Experimental Medicine* in July 1929. Avery now had no alternative but to face Griffith's discovery.